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DEPARTMENT OF THE ARMY  
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## CHEMILUMINESCENCE IN ORGANIC COMPOUNDS

Following is a translation of an article by Dr. Heinz Stork, in the German-language periodical Chemiker-Zeitung Chemische Apparatur, (Chemists' Journal Chemical Apparatus), Vol 85, No 13, 5 July 1961, pages 467-473.

The manifold group of luminescence phenomena may be divided into six types, according to their respective differences in origin. Numbers in brackets refer to similarly numbered items in Bibliography at end:

1. Electroluminescence, which may be observed under the influence of electrical fields with changing polarity (e. g. on ZnS);
2. Photoluminescence, which is incited by absorption of light (fluorescence, phosphorescence);
3. Triboluminescence, which may be traced to mechanical causes (e.g. pulverization of sugar crystals);
4. Crystalloluminescence, occurring during crystallization of certain substances (e.g.  $\text{As}_2\text{O}_3$ );
5. Sonoluminescence, observable in the ultrasonic field (e.g. in electrolytic solutions containing argon);
6. Chemiluminescence

The energy of the radiation emitted in chemiluminescence originates from a chemical reaction; it may be interpreted as the reversal of a photochemical reaction: in the same manner as the radiation absorbed in a photochemical process will entail a chemical reaction, the analogous is true in reverse in the case of chemiluminescence where radiation is a consequence of a chemical reaction. In both cases the

following important limitation will apply: the ensuing process must not be caused by an increase in temperature resulting from the primary (source of) energy. In a true photochemical process, for example, the chemical reaction will not occur because of a temperature increase (caused by incidence of radiation) may have stimulated a certain percentage of the molecules (according to Maxwell's distribution theorem) to an energy level permitting the subsequent reaction; each reacting molecule must receive its stimulation in one single stage of absorption. In analogous manner, the emission of light during chemiluminescence must not occur as a consequence of a temperature increase but the energy emitted by a particle must have been absorbed by the latter from one single impulse. These impulses are given by molecules generated during the particular reaction whose energy has not yet been depleted by collisions with other particles incapable of radiation. The cause of chemiluminescence, in other words, is the transference of reaction energy to atoms or molecules capable of radiation; shown in a schematic formulation:



The particle with a higher energy level ( $A^*$ ), originating from the chemical reaction, will transfer its energy to the particle B which, under emission of light ( $h\nu$ ) will return to the low energy level state. The following substances may act as agent B:

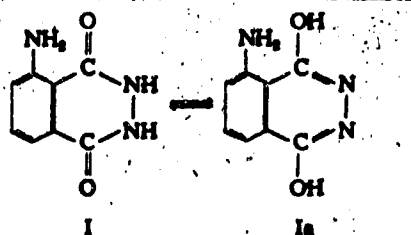
1. One of the reaction partners (e.g. the D-line of the sodium spectrum is emitted in the reaction of sodium vapor with chlorine under reduced pressure [I, 2, 3]);
2. An intermediate substance occurring during the course of the reaction (e.g. in the oxidation of phosphorus and  $P_2O_3$  to  $P_2O_5$  the spectrum of  $P_2O_3$  is emitted [I, 4, 5]);
3. A product of reaction (e.g. only  $CO_2$  bands-i.e., spectral lines - occur in the carbon monoxide-oxygen flame [I]);
4. A foreign admixture (e.g. if oxidisilin is oxidized with permanganate in the presence of the dyeing agent Rhodanite B, strong red luminescence will be observed; luminescence will be weak if the coloring agent is not present in the reaction [I, 6, 7]).

One of the first authors having performed extensive research work in the field of chemiluminescence of organic compounds is B. RADZISZEWSKI [8, 9, 10]. Among other phenomena he observed luminescence upon entrance of oxygen into alkaline solutions of sebatic acids, aldehydes, amides and alcohols; particularly bright luminescence occurs during the alkaline oxidation of Lophine and Amarine. M. TRAUTZ [11] published a comprehensive historical survey of chemiluminescence phenomena and has himself observed luminescence during oxidation of numerous aldehydes, alcohols, phenols and sebatic acids; luminescence is especially intensive in the joint oxidation of aldehydes and phenols. He verifies luminescence of Amarine and Lophine in alkaline  $H_2O_2$  solution; the same phenomenon is observed in Chrysené, Anthracene and Carbazole. TRAUTZ has tested the influence of the oxidation agent on the chemiluminescence in a great number of experiments; bromine water proved to be a particularly effective reagent, showing its greatest effect on heterocyclic compounds containing nitrogen (e.g. quinoline and its derivatives); bright luminescence was generated in the latter. A number of publications [12, 13, 14] deals with the chemiluminescence of Grignard type compounds, occurring during their reaction with air, oxygen and other oxidizing agents. The most noteworthy contributions are those published by W. V. EVANS, R. T. DUFFORD, D. NIGHTINGALE and their colleagues [15, 16, 17, 18]. Chemiluminescence in approximately 200 Grignard compounds is compared by these authors. Nearly all magnesium compounds tested showed luminescence, with the exception of such compounds where magnesium is bonded to a short alkyl remainder. Capacity for luminescence increases in proportion to molecular weight; it is generally greater in the bromides than in the chlorides which, in turn, surpass the iodides. In the phenyl-magnesium compounds, m-substitution was found to have a favorable effect, while an o-substituent will have an even better and a p-substitution the best result. The compound with maximal intensity of luminescence proved to be p-chloride-phenyl-magnesium bromide. No luminescence was shown by organic zinc and mercury halides. Attention is also devoted by these authors to the relationship between chemiluminescence and fluorescence. According to their statements fluorescence was observed in the oxidation products of numerous Grignard compounds (in the section of the long ultraviolet rays), even such substances which had shown no luminescence. Spectral distribution of fluorescence differs from that found for luminescence.

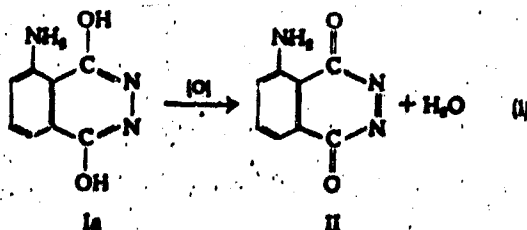
Another phenomenon of chemiluminescence which had been treated repeatedly [19, 20] is the bright green light occurring during alkaline oxidation of N,N'-dimethyl-diacridylic

salt. H. KAUTSKY and H. H. KAISER [21] proved that the spectrum of luminescence is identical with the spectrum of fluorescence of the resulting oxidation product, i.e., N-methyl-acridone.

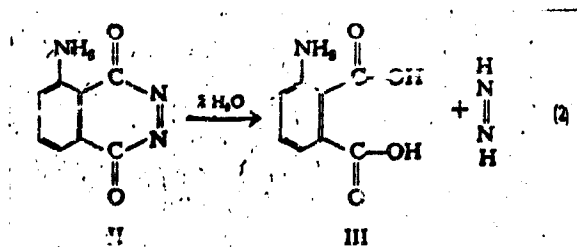
All luminescence phenomena listed thus far are surpassed by far in regard to intensity of luminescence by the cyclic 3-amino-phthalic acid hydrazide, called Luminol (I). This luminescence was discovered by W. LOMMEL in the Bayer factory laboratories at Leverkusen; H. O. ALBRECHT, upon LOMMEL's recommendation, conducted a thorough investigation of this phenomenon [22]. He found that (I) will show intensive blue fluorescence in diluted acid solution; sour solution, however, will not show luminescence when adding NaClO solution. Conversely, (I) will show no fluorescence in alkaline solution, but will emit intensive blue light upon addition of NaClO solution and still stronger intensity will be obtained when adding H<sub>2</sub>O<sub>2</sub> and NaClO solutions. Luminescence will still occur after dilution of the solution to 1/10<sup>7</sup> the original value; its spectrum is not identical with the spectrum of fluorescence. ALBRECHT ascribes the latter fact to changes occurring in the 3-amino group during oxidation. Equilibrium between the two tautomeric forms of (I) will be moved toward the left in alkaline and toward the right in acid solution.



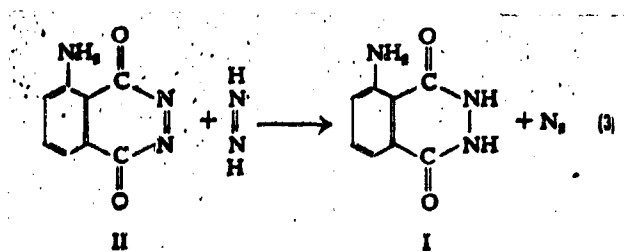
This indicates that fluorescence is attributable to the cyclic hydrazide (I), while luminescence is effected by the dihydroxy compound (Ia). Consequently, ALBRECHT bases his formulation of the reaction process causing luminescence on (Ia), where the first step is shown to be oxidation to form the azodiscyclic compound (II):



(II) quickly hydrolyses in alkaline solution, forming 3-amino-phthalic acid (III) and diimine:



Diimine then reacts with one molecule of (II), which causes re-formation of (I):

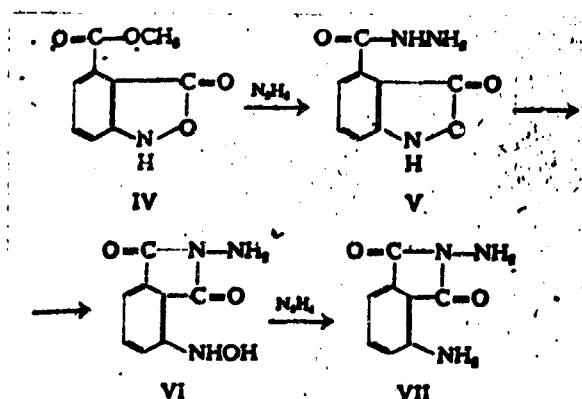


This Luminol molecule is rich in energy and is converted to a less energized stage under emission of light. An important experimental foundation for this reaction path is given by the experiments conducted by H. KAUTSKY and K.H. KAISER [23]. These authors agitated (I) with Ca(OCl)<sub>2</sub>, under acetone, and obtained a violet-red solution displaying the characteristics of an azodizcyclic compound; it emits bright light upon addition of a watery, diluted alkaline solution. A solution of (I) in acetone, however, does not show luminescence when alkaline solution is added; an additional oxidizing agent is required. Luminescence, therefore, is obviously preceded by formation of the azodiacyclic compound and its hydrolyhydrolysis. In accordance with equation (3), re-formation of (I) then takes place which, upon addition of more oxidation agent, again permits luminescence until, finally, all of (I) will have been converted to 3-amino-phthalic hydrazide.

An attempt to classify the chemiluminescence of (I) in the above schematization (in accordance with the reaction mechanism stated by ALBRECHT) will show that it occupies a

special position in this respect. In this case we will not find a reaction product (A\*) which is rich in energy, transferring a portion thereof (by impulse) onto a particle B which thus becomes capable of emission; the particle A\* is here identical with B\*. ALBRECHT interprets luminescence in strongly diluted solutions as a verification of his mechanism, since under those circumstances a transfer of energy by means of impulse is practically impossible.

GLEU and PFANNSTIEL <sup>[24]</sup> have worked on the luminescence of Luminol derivatives. While LOMMEL had used 3-amino-phthalic imide in reaction with watery hydrazide for the production of Luminol, the latter authors' synthesis is based on benzisoxazolon-4-carboxylic acid-methyl ester (IV) which they had been the first to produce:



The ester (IV) is boiled shortly with an excess of hydrazine which will cause its transformation to the hydrazide (V) which, in turn, isomerizes to N-amino-3-hydroxylamino-phthalic amide (VI). By means of reduction with an excess of hydrazine the N,3-diamino-phthalic imide (VII) is formed which is denoted as "yellow hydrazide", according to its color. (Note: GLEU and PFANNSTIEL have pointed out that, according to a private note received from Dr. RAST in the BAYER factory laboratories at Leverkusen: Dr. RAST permitted watery hydrazine to act upon 3-amino-phthalic imide, at temperatures below 75° Celsius.) This substance shows neither fluorescence nor chemiluminescence. Boiling with concentrated hydrochloric acid will transform the yellow product into the isomeric, stable "white hydrazide", i.e., Luminol:

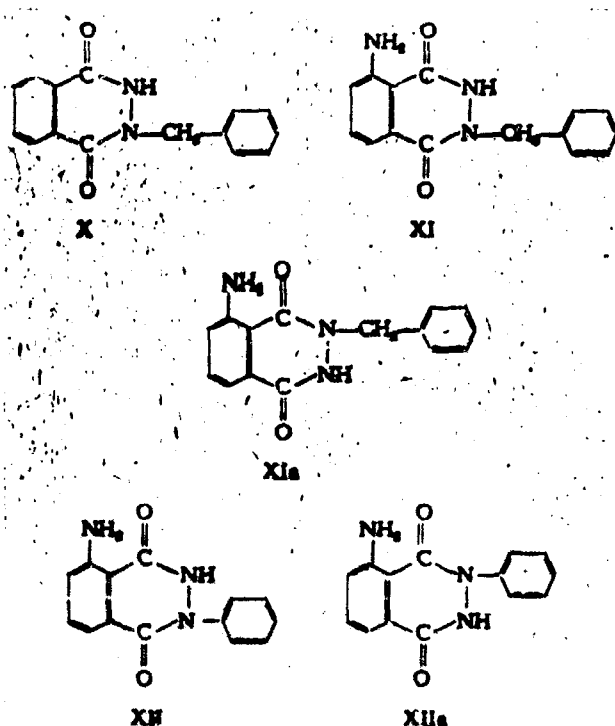


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luminescence as proof of the theory. For this reason he let 3-nitro-phthalic acid react with phenylic hydrazine and, after subsequent reduction, obtained a mixture of the isomeric N-phenyl-3-amino-phthalic acid hydrazides (XII). These compounds also display a clearly distinguishable emission of light, with  $H_2O_2$  addition; despite a strong decrease in intensity of luminescence, noted after the substance had been purified repeatedly, the author considers this luminescence an indication that phthalic acid hydrazides with one substitution having been made at a nitrogen atom will still show emission of light.



In addition to the above quoted compounds, showing substitution in the hydrazine residue, WEGLER produced the dicarboxylic acid hydrazides listed in Table 1; he conducts a comparative study of their luminescent capacity in  $H_2O_2$  alkaline solution, in the presence of hemin.

Brightest luminescence in all these substances is shown by (I); the isomeric 4-amino compound shows equal intensity. XIV, without hemin, shows an intensity equal to that of I with hemin, but no increase in intensity is obtained in XIV after addition of hemin. In a similar manner it is impossible to achieve a catalytic increase in the luminous intensity of XVIII, in spite of the fact that this substance emits a

Table 1

Chemiluminescent cyclic dicarboxylic hydrazides,  
according to R. WEGLER [25]

---

	3-amino-phthalic acid hydrazide
XXI	4-amino-phthalic acid hydrazide
XXIV	Diazonium salt of I
XV	3-hydroxy-phthalic acid hydrazide
XVI	3,6-dihydroxy-phthalic acid hydrazide
XVII	3,5-diamino-phthalic acid hydrazide
XVIII	3-hydrazino-phthalic acid hydrazide
XIX	4-bromo-phthalic acid hydrazide
XX	phthalic acid hydrazide
XXI	Pyridine-2,3-dicarboxylic acid hydrazide
XXII	Anthraquinone-2,3-dicarboxylic acid hydrazide
XXIII	Quinoxaline-2,3-dicarboxylic acid hydrazide
XXIV	Dimethyl-maleinic acid hydrazide
XXV	Diethyl-malonic acid hydrazide
XXVI	Succinic acid hydrazide
XXVII	Diamino ,romellithic acid hydrazide

---

brighter light with  $H_2O_2$  alone, than I under identical conditions. XV shows more intensive luminescence than the unsubstituted compound XX, but less intensive than I; XVI and XVII will not even reach the intensity of the mono-hydroxy compound XV. XX and XXI show equal intensity, both of them surpass XXII. No comment is made concerning the luminescence intensity of the monocyclic hydrazides XXIV, XXV and XXVI; luminescence of the dihydrazide XXVII is stated as being hardly perceptible.

E. S. VASSERMANN and G. P. MIKHLUKHIN [26] report on another group of luminescent hydrazides:

Table 2

Chemiluminescent cyclic dicarboxylic acid hydrazides  
according to E. S. VASSERMANN and G. P. MIKHULKIN [26]

---

XXVIII	Naphthalic acid hydrazide
XXIX	N-amino-phthalimide
XXX	4-nitro-phthalic acid hydrazide
XXXI	Diphenyl-2-2'-dicarboxylic acid hydrazide
XXXII	m-diphenyl-2,2'-dicarboxylic acid hydrazide
XXXIII	cis-delta 3,5-dihydro-phthalic acid hydrazide
XXXIV	delta 2,6-dihydro-phthalic acid hydrazide

---

XXXIII and XXXIV are the compounds displaying most intensive luminosity.

C. N. ZELLNER and G. DOUGHERTY [27] are not content with a qualitative comparison of luminescence intensities, based only on visual perception. They have measured the relative luminescence intensity of 10 phthalic acid hydrazides by means of a Weston photo element connected to a mirror galvanometer with an internal resistance of 896 Ohms. Deflection of the pointer as shown on the galvanometer scale constitutes a measure of light intensity

Table 3

Luminescence of phthalic acid hydrazide derivatives according to C. N. ZELLNER and G. DOUGHERTY [27]

(Measurements were made at two concentrations in 0.25% sodium lye; 0.2 ccm n-NaClO solution were used in each case as oxidation agent. Distance between scale and galvanometer was 1 meter.)

Phthalic acid hydrazide	(a)	(b)
XV 3-hydroxy-(not clean in analysis!)	38.0	65.0
XXXV 3-acetylamino-	28.0	31.5
XXXVI Acetyl-3-acetylamino	11.5	26.5
XXXVII 3-chloro-(not pure in analysis!)	4.0	3.5
XXXVIII Diacetyl-3-benzoylamino-	0.8	1.1
XXXIX alpha-N-methyl-3-acetylamino-	0.0	0.4
XL 4-acetylamino-	9.0	11.0
XLI Acetyl-4-acetylamino-	8.5	10.0
XLII Diacetyl-4-benzoylamino-	0.2	0.2
XLIII alpha-N-methyl-4-acetylamino-	0.0	0.2

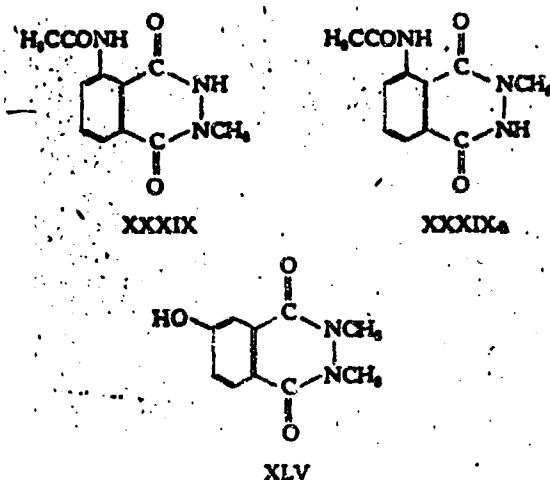
Legend: a) Deflection (cm) (c=0.001 mol/l);  
b) Deflection (cm) (c=0.004 mol/l).

Measuring of the luminescence of Luminol with the apparatus described above was possible only after installing of an additional resistor of 700,000 Ohms in front of the galvanometer: pointer deflection at c=0.004 mol/l was 22 cm. This examination also does not permit a final decision as to

the chemiluminescence of phthalic acid hydrazides with substitution(s) in the hydrazine remainder. XXXVI is formed from XXXV by acetylation; XLI is formed by the same process from 4-amino-phthalic acid hydrazide. There is a possibility that in both compounds the amino group exists in the diacetylated form. In XXXVIII and XLII which are formed from the mono-acetyl-phenylamino compounds by acetylation, at least one of the acetyl groups must be bonded to one of the N-atoms of the hydrazine rest. Luminescence in these compounds is, however, of such extreme weakness that it may be attributable to a lack in purity. The same is true of the compounds XXXIX and XLIII which are obtained from the respective acetylaminophthalic acid anhydrides and methyl hydrazine, together with the isomeric beta-compounds (e.g. XXXIXa) (Positioning of the N-methyl group within the alpha and beta derivatives was not ascertained). Isomeric compounds, when contained in a mixture, may be separated by means of fractionized crystallization. Luminescence of alpha-N-phenyl-4-acetylaminophthalic acid hydrazide (manufactured as number XLIV) could not even be measured with the apparatus used in these tests.

Through oxidation of the hydrazides with NaClO solution and measuring of the nitrogen quantities generated within certain time intervals the so-called oxidation rate may be determined; a certain relationship was found to exist between the latter and the luminescence intensity. All substances with low luminescence displayed a (more or less marked) deceleration in their nitrogen generation; an extreme case was presented by the dimethyl compound XLV which neither showed luminescence nor nitrogen generation. On the other hand, a high oxidation rate does not constitute a criterion for occurrence of luminescence; e.g. no light emission is shown by the nitro-phthalic acid hydrazides, despite the fact that their oxidation progresses as rapidly as that of Luminol.

ZELLNER and DOUGHERTY included in their investigation the amino-benzoyl hydrazides and showed that open hydrazides may also display luminescence; o-amino-benzoyl hydrazide shows well visible light emission, while that of the m-derivatives is hardly perceptible and the p-derivative emits no light at all. Further examples of open hydrazine derivatives showing luminescence are given by isonicotinic acid hydrazide, N,N'-di-isonicotinic acid hydrazine and the N-isonicotinic acid-N'-nicotinic acid hydrazine; these compounds show rather intensive luminescence, according to J. KROH and J. LUSCZEWSKI [287], in the presence of hemin.



K. H. DREW and his colleagues have reported, in a number of publications, on the manufacture and testing of numerous phthalic acid hydrazides containing substitutions [29, 30, 31, 32, 33, 34]. They investigated the influence of the substituents in the benzol ring as well as in the hydrazine rest and have developed a new theory on the mechanism of the luminescence reaction, supported by their experiments. Table 4 shows the results of a comparison of 19 derivatives of phthalic acid hydrazide containing substitutions in their benzol rings.

Table 4

Luminescence of phthalic acid hydrazide derivatives,  
according to K. H. DREW and colleagues [32]

(Luminescence intensities were compared in a Karrerporitsky photometer with the optimal conditions for Luminol (0.04 mol/l hydrazide, 12 x molar quantity NaOH, 4 drops of 6% H<sub>2</sub>O<sub>2</sub> solution and 6 drops of 1% solution of hemoglobine in 0.1% n-sodium lye). First measurement was made at 18° Celsius with hemoglobine addition; the compounds have been arranged in order of decreasing light intensity (column 3). A second test measurement at 100°C without catalyst yielded the results represented in column 4.)

Phthalic acid hydrazide	Color of light emitted	with	without
		hemoglobin 18 C	100 C
I 3-amino	blue	1	4
VIII 3-methylamino	green-blue	2	2
XLVI 3,6-di-acetyl-amino	greenish	3	1
XV 3,hydroxy	blue-violet	4	3
XVIII 3-hydrazino	blue	5	7
XLVII Pyromellithic acid dihydrazide	yellow	6	5
XXXV 3-acetylamino	green-blue	7	6
XIII 4-amino	blue	8	8
XLVIII 3,6-dichloro	blue-violet	9	9
XXXVII 3-chloro	blue-violet	10	11
XL 4-acetylamino	blue	11	13
XLIX 3,5-diamino(*)	white-blue	12	12
L 3-benzoylamino	blue	13	14
LI 3-(beta-sulfo)-hydrazino (sodium salt)	white-blue	14	18
XX Phthalic acid hydrazide	white-blue	15	10
LII 4,5-diamino(*)	blue-violet	16	16
LIII 3-nitro(*)	white-yellow	17	15
LIV 4,5-dichloror	blue-violet	18	17
XXX 4-nitro (*)	white-yellow	19	19

(\*) Solutions of these substances are strongly colored; light intensity values noted in these cases are too low, due to autoabsorption.

The most important results from these comparisons are as follows:

a) o,p directing groups ( $-\text{NH}_2$ ,  $-\text{NHCH}_3$ ,  $-\text{OH}$ ,  $-\text{NHNH}_2$ , and also-somewhat weaker-Cl) will increase luminescence intensity; m-directing groups will have a contrary effect ( $-\text{NO}_2$ , and  $-\text{COCH}_3$ ,  $-\text{COC}_6\text{H}_5$ ,  $-\text{SO}_3\text{Na}$  in the side chains);

b) Double substitution (disubst.) in 3,6-position will show a better effect than single substitution in only one of these positions. (The fact that XLIX forms an exception is due to autoabsorption in the red solution);

c) Substitution in the 3 and 6 positions shows a greater effect than in the 4 and 5 positions.

d) Luminescence is strongly increased by a second hydrazide ring in the molecule.

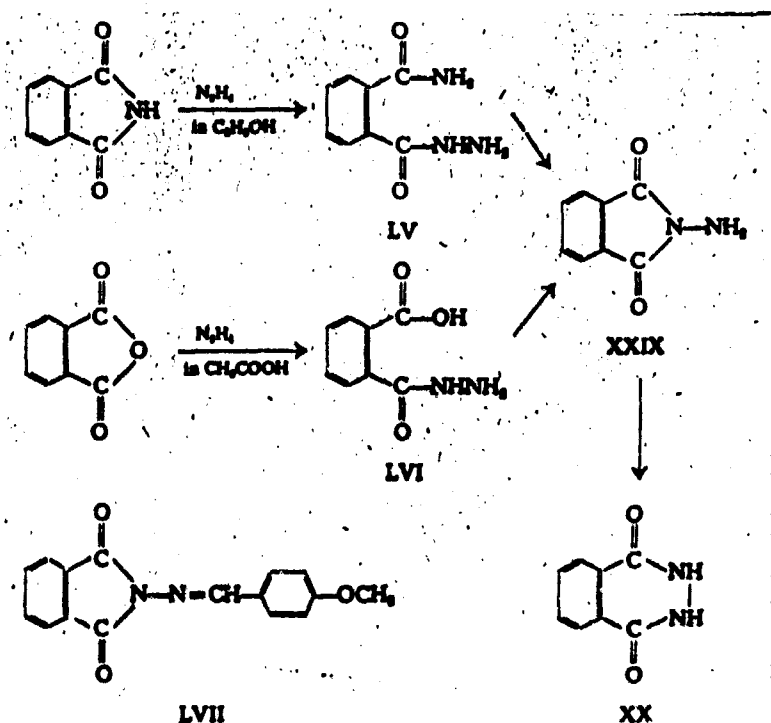
e) Order of luminescence intensity is temperature dependent.

In addition to the comparison of luminescence values the authors have paid particular attention to the methods of synthesis (production); their reports contain a great number of exact preparative instructions showing the most feasible approach for the manufacture of many of the phthalic acid hydrazide derivatives. All syntheses are based on imides and anhydrides with respective substitutions. During reaction of hydrazine and phthalic imide as well as phthalic acid anhydride, N-amino-phthalic imide (XXIX) will form as an interim stage (via LV or LVI, respectively); after prolonged boiling it will be converted to the stable isomeric form (XX). Reversal of this procedure can only be effected through a carbonyl group; in this manner XXIX and XX will be converted (with p-anisaldehyde) into hydrazone (LVII) <sup>/297</sup>. Frequently, only one of the isomeric forms imide in reaction with hydrazine, for example, will only yield the hydrazide XV; 3,6-dichloro phthalic imide will produce only N-amino-3,6-dichloro-phthalic imide. 3-chloro-phthalic imide with 1 mol hydrazine will form N-amino-3-chloro-phthalic imide, but with excessive hydrazine will produce the hydrazide XXXVII; the same reaction is shown by 3-amino-phthalic imide, 3-methyl-amino-phthalic imide, -3-hydrazino-phthalic imide and by the sodium salt of the 3-(beta-sulfo)-hydrazino-phthalic imide. Substituted phthalic acid anhydrides show a strong tendency toward formation of the six-numbered heterocyclic ring. While only N-amino-3,6-dichloro-phthalic imide will be formed during reaction between 3,6-dichloro-phthalic acid anhydride and 1 mol of hydrazine, it will be possible to obtain the hydrazide XLVIII by increasing the hydrazine quantity to more than 2 mols. Tetrachloric phthalic acid and its anhydrides, on the other hand, will produce only N-amino-tetrachloro-phthalic imide, regardless of the hydrazine quantity used. This indicates that some substituents (-Cl, -NH<sub>2</sub>) will impede formation of the six-numbered ring if they are placed in the o-position during ring linkage. No such effect is observed in the m-position; XII and LIV are formed from 4-amino-phthalic imide or 4,5-dichloro-phthalic imide, respectively, with only 1 mol of hydrazine.

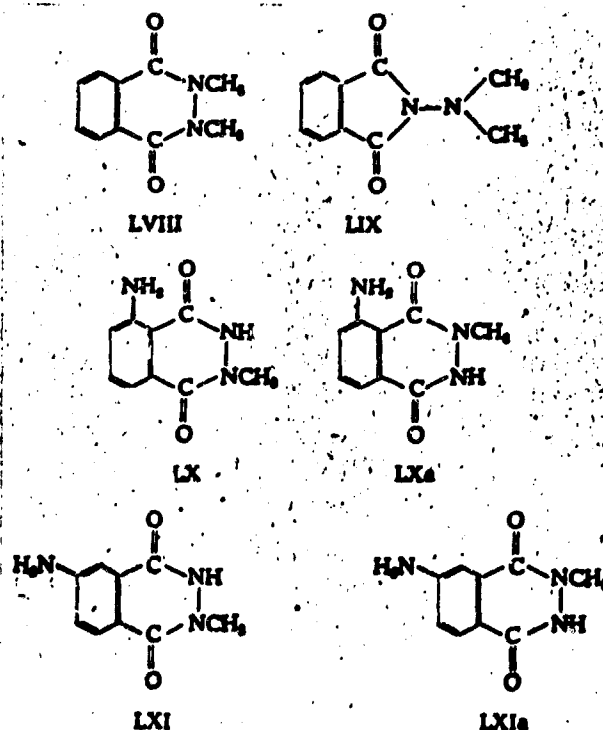
The best approach for obtaining phthalic acid hydrazides with substitutions in the hydrazine rest is presented by the reaction of phthalic acid anhydride with hydrazine derivatives <sup>/317</sup>; N,N'-dimethyl-hydrazine, e.g. will produce LVIII and N,N'-dimethyl hydrazine LIX. In the reaction of methyl hydrazine with 3-nitro- or 4-nitro-phthalic acid anhydride, respectively, separation of the isomeric mixture

through fractional crystallization and subsequent reduction the isomeric pair LX and LXI will be obtained. Positioning of the methyl group could be determined via the copper salts of the diazo compounds generated from LX and LXa by means of diazotization and coupling with beta-naphthol [33].

Like all the other compounds produced (containing the five-numbered heterocyclic ring) LIX showed no luminescence, neither did the isomeric dimethyl compound LVIII (N-amino-phthalic imides also show no fluorescence, even when derived from the fluorescent phthalic imides. All phthalic imides with an amino group in the "3" position show fluorescence, the same group in "4" position will cause absence of fluorescence. Lack of fluorescence in N-amino-phthalic imides is not attributable to the losing of the imide hydrogen but is a characteristic effect of the N-amino group; N-methyl- and N-phenyl-phthalic imide will show fluorescence).



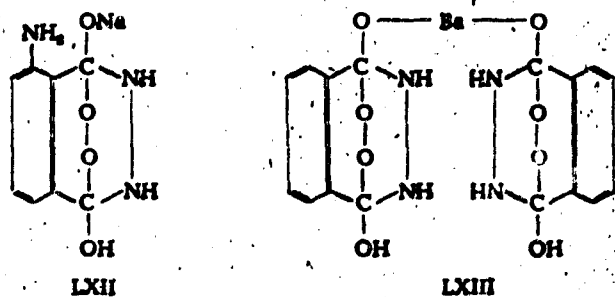
The four nitro compounds corresponding to the mono-methyl compounds LX, LXa, LXI and LXIa also show no luminescence, while LXI was the only amino compound for which non-luminescence was clearly established. The other three amino compounds showed weak but distinctly discernible luminescence. According to the authors' view this luminescence may be



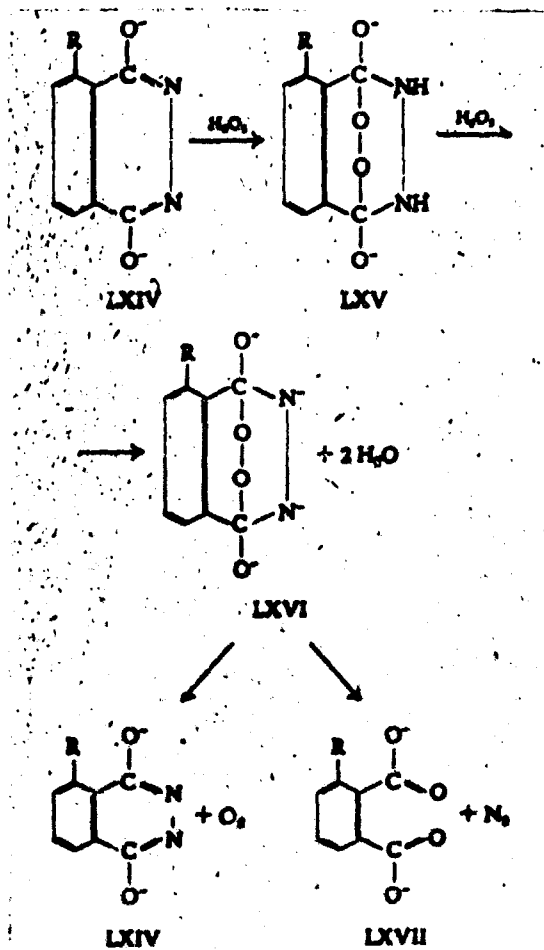
traced to impurities caused by matter not containing methyl which, in turn, may have resulted from traces of unsubstituted hydrazine in the methyl hydrazine. For the purpose of verification of this theory LX was converted into its acetyl form, re-crystallized 8 times while in this form and thereafter the acetyl group removed by hydrolysis; the product when thoroughly purified in this manner showed no further luminescence. The obvious prerequisite for occurrence of luminescence thus seems to be the fact that both nitrogen atoms in the hydrazine rest be free from substitutions.

In the luminescence mechanism theory established by H. D. DREW and R. F. GARWOOD <sup>[34]</sup> a peroxyde is substituted in place of the azoidacyl shown by ALBRECHT. The existence of such peroxyde derivatives of cyclic hydrazide is proven by the isolation of LXII and LXIII in relative pure form. A watery solution of LXII (after addition of hemoglobins) will show bright luminescence, which will still be increased after addition of alkaline matter. Evidently, an equilibrium is reached between the peroxyde and its components.

The first step in the reaction would thus be the conversion of the ion LXIV into the peroxyde LXV. The latter (by oxidation of the imide hydrogen) will convert to the stable intermediate product LXVI which will either re-form LXIV



(where oxygen will be separated) or (under generation of nitrogen) will be converted into the anion of phthalic acid LXVII:



This shows that the luminescent hydrazide is not oxidized itself but only serves as the carrier of oxidation of  $H_2O_2$  caused by the latter itself or by other oxidation agents.

This also explains the observation that benzophenon-o.o;-di-carboxylic acid is formed during oxidation of phthalic acid hydrazide with NaClO solution, while only phthalic acid will be produced if the same oxidation is effected by H<sub>2</sub>O<sub>2</sub>.

The great superiority of the luminescence intensity of Luminol compared to that of any of the other hydrazides is shown impressively in the test results compiled by A. A. PONOMARENKO et alii [357].

Dependence of the Luminol luminescence on the pH value has been repeatedly investigated. R. WEGLER [257] already pointed out that a strongly alkaline Luminol solution containing hemin will emit much brighter light than in weak alkaline concentration. Measurements performed by C. N. ZELLNER and G. DOUGHERTY [277] indicate that a maximum of luminescence intensity is reached at a sodium lye concentration of 0.015 n; higher alkaline concentrations will cause the intensity to decrease again. According to A. BERNANOSE [367], nearly linear dependence exists on either side of this maximum located at a pH value of 10.5 between the logarithm of the total light emission and the pH value. A. A. PONOMARENKO et alii [377] are even using the luminescence beginning at a pH value of 8 to 8.5 as an indicator in acidimetric titration.

Table 5

Luminescence of phthalic acid hydrazide derivatives,  
according to A. A. PONOMARENKO et al. [357]

(Comparison of luminescence values was made with a colorimeter, where the intensity shown by substance I was set equal to 100. Column 2 shows the pH value above which luminescence was observed.)

Phthalic acid hydrazide	Minimal pH value	Relative intensity
I 3-amino-	8.0 -- 8.5	100
XIII 4-amino-	7.7 -- 7.8	4.5
LIII 3-nitro-	8.4 -- 8.5	12.0
XXX 4-nitro-	8.4 -- 8.5	1.5
XXXVII 3-chloro-	8.8 -- 9.2	0.22
LXVIII 4-chloro-	--	0.08
LIV 4,5-chloro-	8.5 -- 8.7	0.20
LXIX Tetrachloro-	9.0	0.53

Intensive study was devoted to the influence of numerous catalysts on luminescence intensity. H. O. ALBRECHT [22] had already observed that luminescence will be intensified by simultaneous action of  $H_2O_2$  and  $NaClO$  or of  $H_2O_2$  and  $K_2[Fe(CN)_6]$ ; he also reported on the intensification caused by blood. K. GLEU and K. PFANNSTIEL [24] noted that crystalline hemin surpasses blood by far in effectiveness and causes a brighter and more prolonged emission; R. WEGLER [25] reports the same results for radish and horse radish juice. Many other ferruginous complex compounds besides hemin have been shown to have a favorable effect on luminescence, e.g. hemoglobin [38], Ferritin and methemoglobin [39], Iron-chlorophyll derivatives [40], iron-phthalic cyanine [38], the inner iron complex salts of phthiocol [41] and the azomethines resulting from substituted salicylic aldehydes and ethylene diamine [42]. TOKUICHI TSUMAKI et al. [43], therefore, recommend utilization of the positive influence on Luminol luminescence as a constitutional criterion for inner complex salts. - Other compounds examined, e.g. copper-ammonia compounds [44], ruthenium chloride and vanadium sulphate [45],  $Fe^{++}$ -,  $Mn^{++}$ -,  $Co^{++}$ - and  $Co^{+++}$ -ions [46] displayed a (more or less) smaller catalytic effectiveness compared to the inner complex salts.

Despite the fact that many of the luminescence phenomena intensified by catalysts have been described as extremely bright and K. PFANNSTIEL [47] has even been able to take photographs in luminescent light, and the actual light quantity yielded during emission is relatively small, as shown by measurements made by L. HARRIS and A. S. PARKER [48]. The authors created a very small zone of reaction (in a very precisely described apparatus), collected the light emitted by this nearly point shaped source by means of a quartz lens and projected this light beam onto the surface of a highly sensitive micro-thermo element which was connected to a galvanometer. The galvanometer-thermo element system was calibrated by means of standard light sources. Maximal yield in light quanta measured with this method amounted to approximately 0.3%. Thorough investigation of agreement between the spectra of luminescence and fluorescence will yet have to be carried out. Success of this research project will probably depend to a large extent on the choice of the correct catalyst. It should be pointed out in this connection that not only the intensity but also the duration of luminescence is increased by some catalysts which fact may provide a temporal margin sufficient for the measuring of spectral distributions. A catalyst of particular suitability might be the inner cobalt-(II)-complex salt of phthiocol: it causes intensive luminescence for a duration of 3 hours [41]. Luminescence will continue even for several days under the influence of radish and

horse radish juices /257.

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